# Fast Quantitative Detection of Cocaine in Beverages Using Nanoextractive Electrospray Ionization Tandem Mass Spectrometry

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Without any sample pretreatment, effervescent beverage fluids were manually sprayed into the primary ion plume created by using a nanoelectrospray ionization source for direct ionization, and the analyte ions of interest were guided into an ion trap mass spectrometer for tandem mass analysis. Functional ingredients (e.g., vitamins, taurine, and caffeine, etc.) and spiked impurity (e.g., cocaine) in various beverages, such as Red Bull energy drink, Coco-cola, and Pepsi samples were rapidly identified within 1.5 s. The limit of detection was found to be 7 ~ 15 fg ($S/N = 3$) for cocaine in different samples using the characteristic fragment ($m/z$ 150) observed in the $MS^3$ experiments. Typical relative standard deviation and recovery of this method were 6.9% ~ 8.6% and 104% ~ 108% for direct analysis of three actual samples, showing that nanoextractive electrospray ionization tandem mass spectrometry is a useful technique for fast screening cocaine presence in beverages. (J Am Soc Mass Spectrom 2010, 21, 290–293) © 2010 Published by Elsevier Inc. on behalf of American Society for Mass Spectrometry

Cocaine (benzoylmethyl ecgonine, MW 303) is a well-known addictive drug, which induces a strong psychological dependence, leads to mental disorders, and results in physiological damage, lethargy, psychosis, depression, even death [1, 2]. Cocaine is restrictedly used for narcotism and abirritation in the hospital; however, it is abused for nonmedicinal and non-government sanctioned purposes in virtually all parts of the world. Recently, the finding that a large number of Red Bull energy drink products with cocaine are on the market [3] has drawn concerns about food safety from international audiences.

Beverages, especially energy drinks are becoming more and more popular, particularly for active people, because of their energy-giving properties. Usually these drinks are composed of several nutritional ingredients, such as vitamins, taurine, and caffeine in various complex matrices, which make these samples difficult for rapid analysis. Protecting people from inferior foods on the market requires novel techniques for fast, sensitive, and specific detection of trace amounts of contaminants in foods and drink. GC-MS has been recommended by the U.S. Food and Drug Administration (FDA) for the detection of cocaine and metabolites in urine [4]. Alternatively, several other methods, such as HPLC [5, 6], LC-MS [7, 8], DART-TOF-MS [9], capillary electrophoresis [10], and radioimmunoassay [11] have been used for the detection of cocaine in actual samples. Owing to the complicated matrices, extensive sample pretreatment, such as degassing, extraction, preconcentration, and derivatization, which are time consuming processes, is usually required for actual sample analysis.

Extractive electrospray ionization (EESI) has been applied for direct characterization of complex samples without sample pretreatment [12–16]. Herein, we establish a novel method based on nanoEESI-MS/MS implemented in a commercial LTQ-XL mass spectrometer for rapid detection of trace amount of cocaine in beverages, such as Pepsi, Coca-Cola, and Red Bull energy drinks without any sample pretreatment.

## Experimental

Methanol (AR grade) was bought from Chinese Chemical Reagent Co. Ltd. (Shanghai, China). Deionized water was used for all the experiments. Red Bull energy drink, sparkling Coca-Cola, and Pepsi products were purchased in local food stores. Dilute cocaine hydrochloride methanol solution (1 mg/mL, Fluka Co., Buchs, Switzerland) was properly diluted to make a series of cocaine standards.

The novel nanoEESI is composed of a gasless nanospray emitter (FS capillary, i.d. 50 $\mu$m; o.d. 250 $\mu$m) for generating the primary ions and a disposable sample

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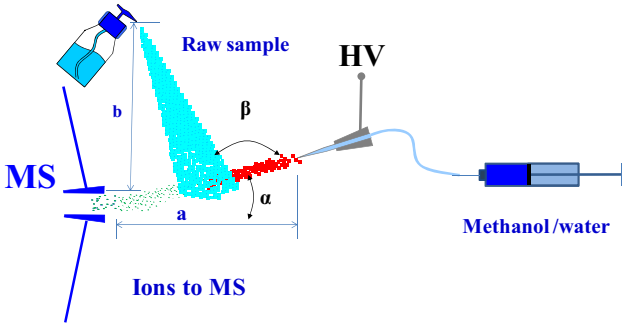


**Figure 1.** Schematic of the nanoEESI setup.

injector for manually introducing the neutral liquid sample (Figure 1). The nanoEESI allows the experiment to be performed without sheath gas or discharge gas, which makes it attractive to in situ analysis of the liquid food samples (e.g., drinks) with complex matrices. The nanospray emitter was placed 4 cm (Figure 1a) far from the inlet of the mass spectrometer. The aerosol generated using the sample nebulizing injector was introduced to the nanoEESI source through a stainless steel nozzle, which was set 6 cm (Figure 1b) way from the ion entrance of the mass spectrometer, and was appropriately fixed to the nanoEESI source, allowing reproducible sample introduction. The angle ($\alpha$) formed between the nanospray emitter and the inlet of the mass spectrometer was 30°; and the angle ($\beta$) formed between the nanospray emitter and the sample injector nozzle was 135°. The temperature of the inlet capillary was maintained at 180 °C during the analysis process. The ion injection time was set to be 100 ms for the LTQ-MS instrument. The contamination of the inlet capillary of the LTQ instrument was significantly reduced by spraying the samples towards the opposite direction of the inlet of the mass spectrometer. A high voltage of +5 kV was supplied to the nanoESI emitter. The primary ions were created by electrospraying methanol/water mixture (1:1, vol:vol) at 0.10 $\mu$L/min. The beverage samples were loaded into a disposable mini spray container (Figure 1), through which 0.1 mL sample was nebulized each time toward the charged plume by squeezing the trigger by using full strength. The sample aerosol lasts about 1.5 s in the nanoEESI source; thus, the mass spectra were recorded using an average time of 1.5 s. Ions of interest were isolated using a mass-to-charge window width of 1.2 unit and then subjected to collision-induced dissociation (CID) experiments performed with 20% collision energy and 30 ms duration.

## Results and Discussion

Red Bull is a famous energy drink containing multiple functional ingredients, such as taurine, caffeine, lysine, inositol, nicotinamide, vitamin B6, and trace amounts of vitamin B12. These compounds were detected with high abundances as protonated molecules at *m/z* 126, 195, 147, 198, 123, and *m/z* 170, respectively, in the nanoEESI-MS spectrum recorded using a blank Red Bull drink sample. The signal intensity of the peak at *m/z* 304 was at the noise levels (Figure 2a). Vitamin B12 at trace levels (3 $\mu$g/250 mL) was also detected as sodiated molecules at *m/z* 1378. Other ingredients, such as glucose, $H_3PO_4$, ammonia, and sodium were also detected from the Red Bull sample (see Figure S1 in the Supporting Information, which can be found in the electronic version of this article, for details of the spectral data). All these assignments were confirmed using MS/MS data listed in Table 1 (the MS/MS data are shown in Figure S1). Once a trace amount of cocaine (1 ppt) was added into the Red Bull sample, a new peak

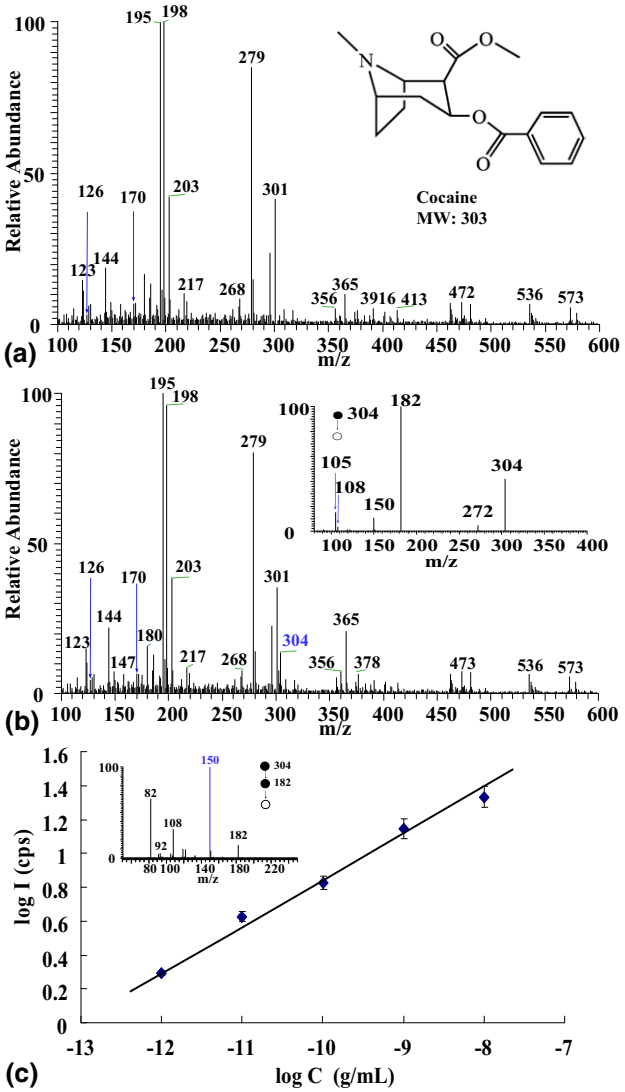


**Figure 2.** Rapid analysis of Red Bull samples using nanoEESI-MS. (**a**) MS spectrum of a blank sample; (**b**) MS spectrum of a sample spiked with cocaine (1 ppt); the inset shows the MS/MS spectrum of protonated cocaine (*m/z* 304); (**c**) linear range for cocaine determination using *m/z* 150. The inset shows the $MS^3$ spectrum of *m/z* 304. The peaks at *m/z* 279, 301, and 144 were known background signals of protonated benzenedicarboxylic acid dibutyl ester (DBP), sodiated DBP, and protonated tripropylamine (TPA).

**Table 1.** Compounds detected from beverages by nanoEESI-MS/MS

| Compounds (MW) | Observed ions | | $MS^2$ product ions *m/z* |
|---|---|---|---|
| | Ionic species | *m/z* | |
| Taurine (125)[a] | $[M + H]^+$ | 126 | 108, 109 |
| Caffeine (194)[a,b] | $[M + H]^+$ | 195 | 138, 110 |
| Lysine (146)[a] | $[M + H]^+$ | 147 | 129, 130, 119 |
| Inositol (180)[a] | $[M + NH_4]^+$ | 198 | 163, 180, 181 |
| Nicotinamide (122)[a] | $[M + H]^+$ | 123 | 106, 95, 81 |
| Vitamin B6 (169)[a] | $[M + H]^+$ | 170 | 152, 134 |
| Vitamin B12 (1355)[a] | $[M + Na]^+$ | 1378 | 1016, 931 |
| Phosphoric acid (98)[b] | $[M + H_2O + Na]^+$ | 139 | 121, 93 |
| Glucose[a]/fructose[b] (180)* | $[M - H_2O + NH_4]^+$ | 180 | 163, 145 |
| Dehydrated fructose (162)[b] | $[M + Na]^+$ | 185 | 167, 149 |
| Biglycan (360)[a,b] | $[M - H_2O + Na]^+$ | 365 | 203, 185 |

[a]Red Bull energy drink.
[b]Pepsi, Coca-Cola soft drink.
*Our MS/MS data were unable to distinguish these two isomers.

showed up at *m/z* 304 in the mass spectrum (Figure 2b). Upon CID, the precursor ions (*m/z* 304) generated ions of *m/z* 272, 182, 150, 108, and *m/z* 105 as the major fragments (inset of Figure 2b). The largest fragment (*m/z* 272) was produced by the loss of methanol from the parent ions; the low abundance, however, suggested this cleavage path was not favored. The ionic fragment (*m/z* 182) observed in the MS/MS experiment was $C_{10}H_{16}NO_2^+$, generated by the loss of benzoyl group from the precursor ions (*m/z* 304). The peaks at *m/z* 150, 108, and *m/z* 105 correspond to the fragment of $C_9H_{12}NO^+$, $C_7H_{10}N^+$, and $C_6H_5CO^+$, respectively. In the $MS^3$ spectrum of *m/z* 182 (inset of Figure 2c), the precursor ions (*m/z* 182) yielded the major fragments of *m/z* 150, 108, and *m/z* 82. These observations were confirmed using reference experiments, for which the dilute cocaine solution (1 ppb) was directly sprayed into the nanoEESI source. The CID data were also in agreement with previous results [17, 18]. Therefore, the experimental data show that trace amounts of cocaine in the drink samples can be rapidly detected using multiple stage nanoEESI mass spectrometry.

Since the matrices were not separated, the characteristic fragment (*m/z* 150) of protonated cocaine observed in the $MS^3$ spectrum was used as the signal for quantification. A dynamic response range of five orders of magnitude was achieved, providing a linear equation $y = 0.2487\ C\ (g/mL) + 3.32$, $R^2 = 0.983$ in double logarithmic scales (Figure 2c). Note that the linear range is up to $10^{-8}$ g/mL although the equation indicates that the signal abundance of *m/z* 150 should be $10^{3.32}$ when 1 g/mL cocaine is used. The relatively narrow linear range was probably caused by the signal fluctuation observed in the $MS^3$ experiments.

This method was further extended to screen the presence of cocaine in Coca-Cola and Pepsi soft drinks. The effervescent sample was immediately sprayed into the electrospray plume without a degassing step once the sample was loaded into the spray container. Similarly, cocaine was detected as protonated molecules at *m/z* 304 from either Coca-Cola or Pepsi samples spiked with low levels of cocaine (1 ppt). Besides the cocaine signals, compounds such as $H_3PO_4$, fructose, dehydrated fructose, etc. (see Figure S2 in the Supporting Information for details of the spectral data) showed up in the nanoEESI-MS spectrum. It is usually a time-consuming process to detect multiple components in sparkling drinks because multiple pretreatment steps must be performed before beverage analysis. For those samples contain high content of gases, an extra step is required to degas the sample before matrices cleaning [19, 20]. As demonstrated here, nanoEESI-MS detects multiple components simultaneously in effervescent drinks without any sample pretreatment, thus providing a useful method for high throughput analysis of drinks.

By directly spraying spiked drink samples ($1.0 \times 10^{-12}$ g/mL) into the ionic plume created by the gasless nanoESI, the ion chromatogram of *m/z* 304 showed instant responses to the presence of sample aerosols. The signal level of *m/z* 304 reached to its 90% height maximum within two scans when the sample aerosol was introduced into the nanoEESI source and dropped down to the background levels within two scans once the sample was removed. No carryover effect was observed during the sample analysis, probably because the sample spray container and the aerosol introduction tube were all replaced for analysis of the next sample. As shown in Figure 3, reproducible signals were obtained for nine measurements using Red Bull beverage spiked with cocaine (1 ppt), providing a reasonable RSD (7.7%, $n = 9$) for fast analysis. Note that a single sample analysis was completed within 1.5 s. The throughput was mainly restricted by the sample-loading step, which took about 5 s for each sample that was loaded using manual operation. Apparently, the fabrication, operation, and maintenance as well have been significantly simplified; thus nanoEESI can be readily coupled to virtually any type of mass analyzer, and can be easily implemented in a miniature mass spectrometer for in situ analysis since it requires no sheath gas (i.e., the heavy gas tank) for sample analysis.

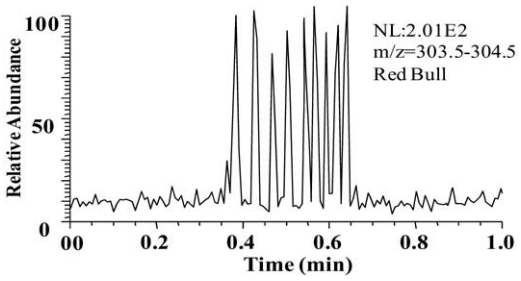


**Figure 3.** The chromatogram of protonated cocaine (*m/z* 304) recorded from Red Bull sample spiked with cocaine (1 ppt). Each peak was recorded with an average time of 1.5 s.

**Table 2.** Analytical data for actual sample analysis

| Sample | Content measured (g/mL)[a] | Values added (g/mL) | Values found (g/mL)[a] | RSD (%)[b] | Recovery (%) | LOD (fg)[c] |
|---|---|---|---|---|---|---|
| Red bull | ~0 | $2.0 \times 10^{-11}$ | $2.1 \times 10^{-11}$ | 7.7 | 104.6 | 7 |
| Coca cola | ~0 | $6.0 \times 10^{-12}$ | $6.5 \times 10^{-12}$ | 6.9 | 107.8 | 15 |
| Pepsi cola | ~0 | $6.0 \times 10^{-12}$ | $6.2 \times 10^{-12}$ | 8.6 | 104.1 | 13 |

[a]Mean values of nine measurements.
[b]RSD deduced from nine measurements ($n$ = 9).
[c]Signal-to-noise ratio was 3 (S/N = 3).

The limit of detection (LOD) of this method was obtained using the most abundant characteristic fragments of $m/z$ 150 observed in the $MS^3$ experiments. Under the experimental conditions, the LOD was 7, 15, and 13 fg ($S/N = 3$) for cocaine in Red Bull, Pepsi and Coca-Cola drinks, respectively, due to the difference in matrices. Acceptable recoveries were obtained for all the three samples, showing that this method can be used for quantification of trace cocaine levels in effervescent beverages. Table 2 summarizes the analytical data.

## Conclusion

NanoEESI, having evolved from EESI, which requires nebulizing gas to produce the reagent ion plume and the neutral sample aerosols, allows proper analytical performance using manual sample introduction with no gas assistance. Neutral samples with complex matrices can be reproducibly introduced into the nanoEESI source, resulting in reasonably low RSD (7.7%, $n$ = 9) for rapid detection of trace analytes. Intrinsically, nanoEESI has advantages, including high sensitivity, good tolerance of matrices, readiness for miniaturization and integration, simple maintenance, easy operation, and low cost. The functional ingredients and/or regulated compounds in various beverages have been rapidly detected using nanoEESI tandem mass spectrometry. The LOD for cocaine was found to be 7 ~ 15 fg for various effervescent drink samples. These results suggest that portable mass spectrometers equipped with nanoEESI sources are suitable for rapid in situ screening regulated compounds such as cocaine in commercially available beverage products.

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## Appendix A Supplementary Material

Supplementary material associated with this article may be found in the online version at doi:10.1016/j.jasms.2009.10.015.

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